THE GASTRULATION OF AMPHIOXUS.

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MATERIAL for a study of the process of gastrulation of *Amphioxus* was collected in 1895 at Faro, Sicily, and at the Stazione Zoologica in Naples.¹ A part of the material was stained at once and surface preparations made; another part, after staining, was imbedded in paraffine, and the remaining eggs were preserved in alcohol.

Kowalevski's and Hatschek's account of the gastrulation left many points still unsettled, and Lwoff's description of the process was so different from those of his predecessors that the entire problem appeared in a new light. Since our work began, no less than three papers have appeared dealing with the gastrulation of *Amphioxus*. It might seem, under these circumstances, that further work would be superfluous; yet the more we have studied the process in *Amphioxus* the more difficult has the problem appeared, and none of these authors seem to us to have reached a satisfactory conclusion.

Methods.

The eggs were preserved in several ways. Corrosive-acetic preparations are best for surface views, and show very clearly, both in surface views and in sections, the dividing and the resting nuclei. Hermann's fluid blackens the embryo so that it cannot be used for surface preparations. The yolk granules come out very clearly in sections, and the cell boundaries are generally very well shown. Flemming's solution — the stronger formula — gives nearly the same results as Hermann's fluid. Embryos fixed by the two latter solutions do not need subsequent staining, although iron haematoxylin will bring out clearly the nuclei, especially those in process of division. After the

¹ For further details, see Morgan ('96).

corrosive-acetic solution the embryos stain well in picro-lithium carmine. Without using the highest powers of the microscope, very little can be made out of the cell-structure of the embryo. Almost all our work, therefore, has been done with a Zeiss immersion 2 mm.

Gastrulation.

One of the great difficulties in following the changes that take place during gastrulation is due to the absence of landmarks. Our attention has been largely directed toward the discovery of points of orientation of the early gastrula. Hatschek relied mainly on the form of the embryo in optical section, and we have also found this, under certain circumstances, a valuable means of orientation. Wilson noticed that the pore at the vegetative pole, which is sometimes left at the end of cleavage, persists occasionally throughout the early period of invagination, and by this means he showed that the vegetative pole was brought into contact with the animal pole; in other words, that the invagination was radially symmetrical. We hoped at first, by using this opening as a fixed point, to determine the later changes that take place, but all traces of the pore soon vanish, except in very abnormal cases.

It is of importance to determine at as early a stage as possible the orientation of the gastrula; and here we have been more successful, since we have been able to distinguish the dorsal and ventral lips of the blastopore at the beginning of the gastrulation process. The gastrula is not perfectly symmetrical, and the same is probably true of the blastula, although more difficult to demonstrate. During invagination the vegetative pole is brought near the animal pole, yet the endoderm turns in such a way that the dorsal side can be distinguished from the ventral. A careful study of the yolk granules, and their appearance in the cells, has aided greatly in orientation. Certain cells, that are turned in at one side, contain relatively fewer and lighter yolk granules, and these cells can be traced from the first stages of invagination until the closing of the blastopore. They mark the dorsal side. As this is a constant feature, it gives a definite means of orienting the embryo.

We have also studied the number and position of the cells surrounding the blastopore at different periods, in the hope of discovering some region of more rapid growth. Finally, the karyokinetic phenomena of the embryo have been carefully examined, for on this process Lwoff has mainly relied. It has been necessary to take all these factors into consideration in order to follow the changes that take place during gastrulation.

Kowalevski and Hatschek have shown that the blastula is composed of larger and smaller cells, and several more recent authors have observed the same fact. MacBride makes the surprising statement that the cells of the blastula wall are all of equal size. He has probably confused cross-sections of the blastula with longitudinal ones, otherwise it is difficult to see how such a mistake could have been made.

The yolk granules almost completely fill the cells around the vegetative pole and gradually diminish in number toward the animal pole.

The gastrulation begins by a flattening of the vegetative part of the egg (Pl. XXXIII, Fig. 1). The lower hemisphere then turns in and slowly obliterates the segmentation cavity (Pl. XXXIII, Fig. 2). The invagination is not entirely radially symmetrical, for the inturned cells bend over more toward one side of the embryo than toward the other (Pl. XXXIII, Fig. 2). At this time the segmentation cavity almost disappears at one side, which becomes subsequently the dorsal side of the embryo, but a portion of the segmentation cavity is left around the remaining part of the inturned cell plate, and, in general, is largest exactly opposite the dorsal side, and in this way a dorsal and a ventral side of the gastrula are distinguishable at an early stage of gastrulation. The outline of the embryo may also be used, as Hatschek demonstrated, to determine points of orientation, as shown in Pl. XXXIII, Fig. 2. The outline of the dorsal side is less rounded than that of the ventral side. This seems to be constant; but, if the sections do not pass dorso-ventrally, this difference cannot be made out.

A further point of orientation is found in the appearance of the inturned cells of the dorsal side. These cells are somewhat smaller than the other invaginated cells, and they resemble

closely the cells of the outer surface of the dorsal side (Pl. XXXIII, Fig. 1). On the other hand, at the ventral lip there is rather an abrupt transition between outer and inner cells (Pl. XXXIII, Fig. 2). MacBride has called attention to these differences, but it is not improbable that in the early stages, at least, he has confused the dorsal and the ventral sides of the gastrula.

Although the embryos can be oriented, as stated above, yet it is still very difficult to determine how the closure of the blastopore is brought about. While the blastopore becomes smaller, the embryo is, at the same time, changing its shape, so that it is not possible to assume that any one point is fixed in relation to the others. Moreover, the possibility of cell-migration must also be kept in mind. Lwoff has considered the presence of karyokinetic division as a criterion of growth; but it must not be forgotten that growth need not follow unless after division the cells increase in size. Furthermore, cell migration is known to take place without cell division.¹

As stated above, we had hoped to make use of the vegetative pore, and by this means to determine how the closure of the blastopore takes place. The pore is most often present in series of eggs that do not develop normally, and hence there is a certain amount of risk in using such a feature to determine the changes that take place in the normal egg. The pore seems generally to disappear in the later stages. Moreover, other openings are sometimes found in the endoderm, due, in some cases at least, to changes in shape of the cells at the time of division. Pl. XXXIV, Fig. 17, shows a cross-section of an egg in which the vegetative pore is large, and lies near the highest point of the invaginated cells.

Occasionally a gastrula is found with the cells around the vegetative pore turned outward (Pl. XXXIV, Fig. 16); and the cells remain in this position even during later stages, as shown in Pl. XXXIV, Fig. 19. This embryo throws some light on the way in which the blastopore closes (Pl. XXXIV, Fig. 19). As it was found among embryos in which, on an average, the

¹ In the sea-urchin the archenteron is formed by cells pushing into the segmentation cavity.

blastopore was nearly closed, the figure shows the relative growth of the dorsal and ventral walls. It will be noticed that the ventral wall is longer than the dorsal, and this is confirmed by other results.

In one case that we have met with, an ectodermal pore was present at the animal pole (Pl. XXXIV, Fig. 18), yet the presence of this pore has not prevented the invagination of the larger cells. If, as seems probable, the opening was present during the gastrulation period, the embryo shows that the process of gastrulation may take place even when a large pore is present in the wall. That this is possible is also shown when the vegetative pore is present, and yet invagination takes place. Therefore, whatever mechanism is invoked to explain the process of gastrulation, the process is of such a nature that it does not demand a closed blastocoel space.

At first the outline of the blastopore is oval (Pl. XXXIV, Fig. 11). A large number of cells bound the opening, but, as there is no sharp line of demarcation between the outer and the inner invaginated cells, and since some of the cells at the edge are partly within and partly without the rim of the blastopore, the exact number and the shape of the boundary cells cannot be accurately determined. In Pl. XXXIV, Fig. 11, about forty-two cells form the rim of the blastopore. At a later stage the number is smaller. In Pl. XXXIV, Fig. 12, about thirty-four cells are around the margin. When the blastopore has further closed (Pl. XXXIV, Fig. 13), twenty-eight cells were counted. Finally, when the blastopore is reduced in size, as shown in Pl. XXXIV, Fig. 14, only ten cells were present.

How can we picture to ourselves the gradual reduction in number of the cells as the blastopore becomes smaller? It appears at times that, as the rim of the blastopore diminishes, certain of the cells continue at the edge, but that others are left behind in the general movement toward the center. In the latest stage the boundary cells are elongated, and as a result a larger number of cells surround the reduced blastopore than would be the case if the cells all retained their earlier form. If we judge by the shape alone of the blastopore, the closing takes

place at nearly the same rate from all points ; at least, until the blastopore is much reduced in size. The outline of the blastopore is generally oval, with the long axis connecting the dorsal and ventral sides. At the time when the embryo is flattened on the dorsal side to form the nerve plate, the blastopore changes its shape, so that it becomes somewhat elongated from side to side (Pl. XXXIV, Figs. 13 and 14).

Karyokinetic figures are found sometimes parallel to the margin (Pl. XXXIV, Fig. 12), sometimes at right angles to it. There is great irregularity in the distribution of the dividing cells. In some embryos a large number of cells are in process of division, in other embryos nearly all the cells are in a resting stage. Sometimes groups of cells may be found dividing and others resting, but such occurrences are exceptional, and, as a rule, the karyokinetic figures are scattered irregularly over the surface.

Lwoff has claimed that at a certain period cell division is more rapid on the dorsal side of the blastopore than elsewhere, and, in consequence, the ectoderm turns in and forms the dorsal wall of the archenteron. A careful examination of a large number of embryos at all stages of development gives no support to this view.

It is important in comparing the changes in the shape of the embryos of different stages to use the same series preserved by the same methods. It is also important that sections in the dorso-ventral plane and in oblique planes be carefully distinguished.

The early gastrula is shallow and saucer-shaped (Pl. XXXIII, Fig. 2). The lips are then brought nearer together, so that the embryo is longer, but correspondingly narrower (Pl. XXXIII, Fig. 3). The embryo continues to narrow dorso-ventrally and also laterally, and at the same time it grows longer. This process continues, and for a time the growth of the dorsal and ventral lips seem to be about equal. In still later stages (Pl. XXXIII, Fig. 5) the ventral wall seems to grow more rapidly ; it bends inward toward the dorsal surface, and extends farther posterior than does the dorsal lip.

Since there are no definitely fixed points, it is very difficult

to determine in what region the bending in of the wall takes place. The method of closing cannot, therefore, be determined by any one criterion, but all the observed results must be taken into account.

It should be noticed that in these figures the dorsal (*d.*) and the ventral (*v.*) lips of the blastopore are, throughout the early

FIGS. I-XX.

stages, equally distant from the point of greatest curvature (*a*). If, on the other hand, the bending took place nearer the dorsal or the ventral lip, one side would be shorter and the other longer; and this is not the case.

Hatschek figures one side — the ventral — as longer than the other side, — the dorsal, — but the dorsal subsequently grows in length until it is approximately the same length as

the ventral; the ventral wall increasing very little, if at all, during the period of closing. A comparison of our Figs. 2, 3, and 5, Pl. XXXIII, shows unmistakably that both the ventral and dorsal walls are longer in the older stages.

A comparison of Hatschek's Fig. 24 with our Pl. XXXIII, Fig. 2, will show that what Hatschek supposed to be the dorsal side is in reality the ventral one.

It is important to keep in view the different ways in which the gastrulation might be interpreted, and we have tried to illustrate in the accompanying text-figures the different possibilities. The first series, I-V, represents a symmetrical closure of the blastopore. The final closure at *f* is opposite the anterior end *c*. The primary axis of the blastula and of the gastrula corresponds with the antero-posterior axis of the embryo. The closure of the blastopore is shown in V, and is seen to take place equally from all points. Kowalevski supposed this to be the way in which the blastopore closes.

The second series, VI-X, represents an unsymmetrical closure of the blastopore. The ventral lip at *a* is supposed to be fixed in VI, and in the successive figures, VII-IX, the dorsal lip is represented as bending over and elongating to close the blastopore. The closure of the blastopore takes place over the dorsal side of the embryo. Fig. X shows the successive stages in the closure of the blastopore. If we compare Figs. V and X, we see that when the blastopore is turned upward toward the observer the point of greatest bending of the wall is opposite the blastopore in V, while in X the point of greatest bending is anterior to the center of the blastopore (see also Fig. VIII). This series, VI-X, represents Hatschek's idea as to the way in which the blastopore closes.

The reverse method of closing is shown in series XI-XV. Here the dorsal lip is represented as fixed, and the ventral as bending over to close the blastopore. Hence the closing is on the ventral side of the embryo.

It will also be noticed that in the last two series (VI-X and XI-XV) the point of greatest bending shifts gradually around the anterior end of the embryo as the closing takes place; while in the first series the point of greatest bending remains fixed.

There are, in fact, two ways in which we may think of the changes shown in Figs. VI-X and XI-XV as taking place. The wall that grows backward to close the blastopore may either simply elongate as a result of cell growth, and the bending at the anterior end remain fixed, or the growth backwards might be due to a gradual shifting of the part of greatest bending along the opposite side; in consequence of this the dorsal wall would increase in length at the expense of the ventral in one case, and the ventral at the expense of the dorsal in the other. It is also possible that both a change in the bending and cell growth might take place at the same time.

In a fourth series we have tried to represent our own idea of the method of closure of the blastopore. In the first figure of this series, XVI, the gastrula is represented as being somewhat unsymmetrical. The dorsal lip is at *c*, the ventral at *a*. The point of greatest bending of the gastrula is near the center, but a little toward the ventral side. At a later stage, XVII, both dorsal and ventral sides of the gastrula have come nearer together, and the embryo has, in consequence, become longer. The dorsal and ventral sides are of about equal length, and the point of greatest bending is opposite the blastopore. In a later stage, XVIII, the walls grow longer and the dorsal side flattens somewhat. The opening of the blastopore is still opposite the point of greatest bending. In the last stage, XIX, the ventral lip grows faster and the blastopore opens more on the dorsal side. Fig. XX shows the method of closure as seen from the posterior side. The series of concentric circles represent the successive stages. The final stage is excentric, owing to the more rapid growth of the ventral lip.

If instead of a dorso-ventral series we had made use of a lateral one, that is, one from right to left, to illustrate our idea of the method of closure of the blastopore, we would have given a series exactly like that drawn in I-V. Even in the dorso-ventral series our idea of the method of closure corresponds more nearly to that of the first series than to any other; the points of difference being: first, the unsymmetrical gastrula at the earliest stage (XVI); second, the flattening of the

dorsal side in XVIII; and, third, the more rapid growth of the ventral wall in the final stage of closure.

Cell Division during Gastrulation.

Cell division occurs throughout the entire period of gastrulation both in the ectoderm and in the endoderm. We have examined a large number of preparations, both sections and surface views, to see whether cell division is more frequent in one part of the embryo than in another. Although cell division is present at all times and in all stages, yet we have found, in general, no region of more rapid cell multiplication.

Great individual differences exist, for while one preparation may show more karyokinetic figures on the dorsal or on the ventral lip of the blastopore, other preparations of the same age may show other regions of cell multiplication. Hence, with a preconceived idea as to the place at which more rapid growth occurs, one could easily find preparations confirming such a view. But an unprejudiced examination shows that cell division is general and not restricted to any special region. Text-figs. XXI–XXVIII show in what regions cell division is taking place in certain embryos of various ages. The drawings are diagrammatic reconstructions of serial sections. The dorsal side is indicated by the parallel lines. One figure of each pair shows, therefore, the dorsal, and the other the ventral side. The dividing nuclei in the ectoderm are represented by solid dots, and those in the endoderm by circles.

Fig. XXI *a, b* represents the embryo at midnight, corresponding in age with the embryo shown in Pl. XXXIII, Fig. 2. Only two ectodermal cells are dividing, while many endodermal cells are in process of division. Another embryo of the same age is shown in Fig. XXII *a, b*. Here the number of cells dividing in ectoderm and endoderm is about the same. The dividing cells are scattered nearly equally throughout the embryo.

The next stage, Figs. XXIII and XXIV *a, b*, represent the embryo at 3.30 A.M. Both show cells dividing around the blastopore, and there is also a region of division at the anterior end.

In one of these the ectoderm cells on the dorsal side and in the other on the ventral side are dividing more rapidly. Other preparations of the same age were found in which the cell division is not more common around the blastopore than elsewhere.

At 4.30 A.M. the embryo has closed in further (Figs. XXV *a*, *b* and XXVI *a*, *b*). In the first of these figures it is

Figs. XXI-XXVIII.

noticeable that cell division is very frequent in the ectoderm of the ventral side. In the other very little cell division is present, and that mostly around the ventral lip of the blastopore.

Figs. XXVII *a*, *b* and XXVIII *a*, *b* at 6 A.M. show, in one case, several endoderm cells and few ectoderm cells in process of division. In the other embryo the reverse is true.

After this stage the blastopore closes in more rapidly from the ventral side, and cell division seems to be more rapid on this side, both in ectoderm and endoderm.

Distribution of Yolk in the Embryo.

At the time when the blastula wall first begins to flatten we have attempted to discover whether the yolk is equally distributed throughout the endodermal plate. In many cases we have found that the embryos preserved in Flemming's or in Hermann's fluid show at one end a region of small cells bearing fewer yolk granules, and these granules are lighter in color than those of the rest of the inturned cells¹ (Pl. XXXIII, Fig. 1). At the opposite end of such a section there is an abrupt demarcation between the large yolk-bearing cells that are turned in, and the smaller and lighter cells outside. This difference in the two ends of the section is shown by many series. In other series, cut more or less at right angles to the preceding, the difference is not seen, of course, in the middle sections of the series, but generally at one end of the series the lighter cells can be found, and at the opposite end the yolk-bearing cells extend to the margin of the blastopore.

This difference in the endoderm we have been able to trace throughout all the subsequent periods of development, and we have been able to show that the region of clearer cells becomes the dorsal wall of the archenteron. Hence the dorsal lip of the blastopore is identified even at the time when the first beginning of gastrulation is evident. It is, therefore, highly probable that even in the spherical blastula there is a bilateral symmetry already present. We have laid more stress on this means of orientation than on any other, and have determined the dorsal and ventral sides in this way.

A later stage in the gastrulation is shown in Pl. XXXIII, Fig. 2. At this time the endodermal plate has bent upward and almost touches the ectoderm. The section is in a dorso-

¹ It is difficult to determine whether the lighter granules are in reality lighter than those elsewhere, or whether it is only an optical effect of the light passing through fewer granules.

ventral plane and shows the difference in the ectoderm at the dorsal and ventral lips of the blastopore. At the dorsal lip the endodermal cells are, as before, lighter and contain less yolk and differ little from the ectodermal of the outer surface; in fact, there is really no histological difference between the cells just outside and just within the lips of the blastopore over the dorsal side. It is to be noticed that there are more of the lighter cells on the dorsal wall than in the preceding stage. This might result either by a continued rolling in of outer cells, or by a multiplication of cells already inside, or by both processes combined, or even by differentiation of the cells anterior to the clear region. It is certain that the endodermal cells continue to divide during development, and we have seen no evidence of an actual inrolling. Whether the division of the cells in this region is, in itself, sufficient to account for the increase in the number of cells is a point almost impossible to determine.

The same section (Pl. XXXIII, Fig. 2) shows also the characteristic shape of the embryo at this stage; over the dorsal side the embryo is less curved than over the ventral side. The deepest part of the invaginated endoderm is somewhat toward the ventral side. The effect produced is that the ventral part of the endoderm is thrown over toward the ventral side of the embryo. Surface views of entire embryos show the same asymmetry in the gastrula. On the ventral side of this section (Pl. XXXIII, Fig. 2) there is an abrupt transition between the large yolk-bearing cells and the smaller cells that continue out into the ectoderm. Whether these smaller cells that are partly turned into the blastopore belong to the endoderm or to the ectoderm can only be determined by their fate. They will be considered later.

There is generally present on the ventral edge a dilatation of the segmentation cavity that is characteristic for this period; on the dorsal side the ectoderm and the endoderm are almost in contact.

An older stage is shown in Pl. XXXIII, Fig. 3. The embryo is now cup-shaped and the archenteron much deeper than before. The deepening is, in part, the result of the

approach of the sides of the embryo, so that it is now narrower dorso-ventrally and also from side to side. As a result, the blastopore becomes smaller (Pl. XXXIV, Figs. 11 and 12) and at the same time the saucer-shaped embryo (Pl. XXXIII, Fig. 2) becomes elongated into a cup-shaped form. The cells are smaller and more numerous in the older embryo (Pl. XXXIII, Fig. 3), but there is no evidence that the change in form of the embryo is the result of cell activity in any particular region. The dorsal wall of the archenteron is covered by lighter cells, and this region is longer than in the preceding stage. The clear cells of the dorsal wall are now larger than the ectodermal cells outside the blastopore, although just at the lips of the blastopore the transition between inner and outer cells is gradual. At the ventral lip the yolk-bearing cells come nearly to the lip of the blastopore and are followed by a few small, rounded cells. The largest endodermal cells bearing the greatest number of yolk granules are found at the innermost part of the archenteron; the cells decrease gradually in size, and in the number of granules contained in them, toward the ventral side.

Around and within the ventral lip of the blastopore, during the early gastrula stages, there are frequently found, as just described, small cells, that contain much less yolk than the large endodermal cells, and closely resemble the ventral ectoderm in appearance (Pl. XXXIII, Figs. 2 and 3). Even in later stages these small cells are often found. We have carefully examined many different series of sections to see if the spherical form of these cells is the result of cell division, but cell division does not seem to be more frequent here than elsewhere, and the nuclei of the cells are often in the resting stage. It is difficult to determine whether any of these cells come to lie eventually in the archenteron, or whether in later stages, as the yolk-bearing endodermal cells multiply, these cells turn out into the ectoderm.

The dorsal side of an embryo (Pl. XXXIII, Fig. 4 *A*) somewhat older than the last is shown in Pl. XXXIII, Fig. 4 *B*. The lighter cells of the dorsal wall are shown also in the figure, and their similarity to the ectoderm outside is evident. The

yolk granules in the ectoderm are fainter and are beginning to disappear. The granules grow fainter in that part of the ectoderm which corresponds to the animal pole. Around the blastopore they persist longer; the cells outside the dorsal lip containing granules similar to those in the cells of the dorsal wall of the archenteron.

An older stage, two hours later than the last, is drawn in Pl. XXXIII, Fig. 5. The embryo has greatly elongated, the blastopore is reduced in size and now opens somewhat on the dorsal surface at the posterior end of the body. The cells over the dorsal wall of the archenteron are broader than those elsewhere; they are also clearer and contain fewer and lighter yolk granules. As much as two-thirds of the dorsal wall is formed by these cells, which pass gradually into the more anterior cells containing more yolk. The cells of the ventral wall are smaller than those at the anterior end and are also smaller and darker than those over the dorsal wall.

The cells of the ectoderm over the dorsal surface are very similar to the endoderm cells over the dorsal wall. The more anterior of those ectodermal cells are smaller, while those near the dorsal lip of the blastopore are almost identical with those within.

On the ventral wall the ectodermal cells are smaller than elsewhere, but they grow larger as they approach the ventral lip of the blastopore. At the sides of the blastopore, also, there are larger cells, so that a ring of large cells surrounds the blastopore.

In the later stages the cells over the dorsal and dorso-lateral walls of the archenteron continue to grow clearer and the yolk in them to disappear. A cross-section of an embryo at 8 A.M. is shown in Pl. XXXIII, Fig. 6. Here the difference between the dorsal and ventral wall is clearly seen. The dorsal surface of the embryo is slightly flattened to form the medullary plate. This section is taken at about the level of the posterior third of the embryo. Sections of the same series taken at the extreme anterior end show that the cells on the dorsal wall contain more yolk and resemble those on the ventral wall.

In the later stages changes take place in the endoderm, bringing about a rearrangement of the cells and also involving

a change in the character of the cells themselves. The anterior end of the archenteron becomes larger, and at the same time the cells covering this portion of the cavity become smaller (Pl. XXXIII, Fig. 7 *A*). This region was at first surrounded by the large dark yolk-bearing cells (Pl. XXXIII, Fig. 5), but these cells seem to shift posteriorly; at any rate, the large yolk-bearing cells are now found on the ventral and ventro-lateral walls of the archenteron at the posterior end of the body.

About this same stage, or a little later, a curious change takes place that has greatly puzzled us. Yolk granules begin to appear in all the cells of the body, and what is most surprising is that the yolk in the large endodermal cells does not seem to decrease in amount. We cannot offer, therefore, any explanation as to the meaning of this phenomenon. It might seem that the change was brought about by a decrease in the volume of the cells, and that they contained the same absolute amount of yolk as before, the change being apparent rather than real. Measurements of the embryo show, however, that it is increasing in size, and the body-walls are as thick or thicker than before. The cells are, it is true, higher and narrower than before, but we do not see how this alone could produce the effect. However, even in the cells of the ectoderm yolk granules reappear in later stages. A longitudinal section of an embryo at 11 A.M. is drawn in Pl. XXXIII, Fig. 7 *A*. A detailed and more magnified part of the dorsal wall (at *x*, in Fig. 7 *A*) is shown in Fig. 7 *B*. The ectoderm that forms the nerve plate contains more yolk granules than in the earlier stages, and the endoderm of the dorsal wall (*end.*) is now also filled with yolk granules. Another part of the same embryo anterior to the last (at *y* in Fig. 7 *A*) is shown in Fig. 7 *C*. In this region the ectoderm contains almost no yolk, but the endodermal cells are filled with it. A section through the ventral wall (at *z* in Fig. 7 *A*) is shown in Fig. 7 *D*. Here the ectoderm contains very little yolk, while the endodermal cells are tall, closely packed together, and filled with yolk granules.

A later stage (Pl. XXXIII, Fig. 8) shows that the yolk granules reappear even in the ectoderm. At this time the first

eight body cavities are given off; the cells that form the notochord, and the mesodermal pouches are filled with yolk granules, and the ectoderm is also. This reappearance of the yolk in the later stages occurs after the blastopore has been closed, and at a time when all the regions of the body are easily distinguishable. What we have stated in regard to the earlier stages, throughout which the yolk has been gradually disappearing from certain cells and remaining constant in others, is not effected by these later changes. We feel, therefore, no less certain of our results, based, as they are, largely on the distribution of the yolk and the histology of the cells during the early stages.

Irregularities in the Endoderm.

In looking for the vegetative pore as a means of orienting the gastrula stages, we soon found that, at other places, depressions occurred in the endoderm resembling the point at which the vegetative pore closes (Pl. XXXIII, Fig. 9). We had, therefore, to abandon this landmark as a means of orientation. Often we have found that irregularities in the endoderm are due to the rounding up of cells during the final stages of division. In other cases, as in Pl. XXXIV, Fig. 20, at *x*, the nuclei are in a resting condition, but this may be interpreted to mean that after the division the nuclei have become spherical, but the cells have not yet lost their rounded contour. This view is supported by the fact that two such cells are generally found together. In the ectoderm, too, we have often seen two dividing cells showing the same tendency to become spherical during division. However, cells in all phases of division may be found that have not rounded up as described, yet even in these cases the inner free ends of the cells are rounded, thus breaking the even contour of the endoderm.

Oblique Sections through Blastopore.

It has been pointed out that the early gastrula is somewhat asymmetrical (Pl. XXXIII, Fig. 2), the invagination being

deeper on the ventral side, making the outline of the embryo more rounded there. A somewhat similar effect is sometimes produced by an oblique section through a slightly elongated embryo. Sections similar to text-fig. XXIX *d* are sometimes obtained, but it is unsafe to judge from such a section alone that the more rounded side is the ventral one. The entire series of sections must be known, *a-f*. During the first stages of gastrulation there is an extraordinary amount of variation in the shape of the embryos. Forms with a somewhat elongated blastopore are not infrequent, and an oblique section of such an embryo produces the effect shown in the figures. Even in the later stages, the same asymmetry may be found as shown in Pl. XXXIII, Fig. 4 *A*. As a matter of fact, the dorsal side of the embryo is in this case the shorter

FIG. XXIX.

one, but other embryos of the same age may be cut in such a way that the shorter side is the ventral one. It would therefore be easy to arrange a series of these forms in such a way as to make it appear that the closure of the blastopore is over the dorsal side. Only after a careful and prolonged examination of both sections and surface views have we been convinced that this condition is the result of an oblique section of a somewhat elongated embryo. If, for instance, the series of sections to which XXIX *c* belongs, be examined, it will be found that at one end of the embryo the sections are completely closed, *a-b*; but at the other end of the series the opening of the blastopore enlarges, as shown in *e, f*.

Sections of the kind, with the dorsal side shorter than the ventral, might seem to confirm Hatschek's view of the closing of the blastopore over the dorsal side, but we hope we have shown this view to be erroneous. Without other means of orientation the shape of the section may be very misleading,

especially when such great variation is present. Sections of embryos preserved in corrosive acetic will not show the differentiation of the dorsal wall upon which we have mainly relied, and after repeated examination of series of sections preserved by this fluid, we have been obliged to give up all hope of orienting with certainty the early gastrula stages. In the later gastrula stages the shape of the embryo is sufficiently characteristic to determine its orientation, but, in the early stages, while in many cases the shape of the embryo might seem to be sufficiently characteristic to determine the dorsal and ventral sides, yet such a criterion alone is very unsafe. We do not hesitate to say that many of the sections figured by some of the more recent authors are in all probability oriented wrongly.

Historical Review.

Kowalevski gave a very brief account of the process of gastrulation in *Amphioxus*. A radially symmetrical invagination is described; the gastrula grows longer, and the small blastopore lies at the posterior end. Later the blastopore shifts somewhat to the dorsal side. More recent writers have inferred from Kowalevski's brief account that the gastrula axis corresponds to the embryonic axis, and while such a conclusion is probably true, yet Kowalevski's description is so very brief that we can only infer this to be his meaning.

Hatschek claimed to have been able to distinguish a bilateral symmetry at the time when the invagination is completed. We have shown that even at an earlier stage the bilaterality is present and is shown by the differentiation in the flat plate that is subsequently turned in. Hatschek noticed that the large yolk-bearing cells around the vegetative pole of the blastula, that subsequently form the endodermal plate, occupy only about one-third of the circumference of the blastula wall, and hence are, at first, too small to fill the entire inner surface of the cap-shaped gastrula. He supposes that increase in the volume of the endodermal cells takes place during invagination, and this increase, he suggests, is brought about by absorption of the fluid of the blastocoel space; yet his figures show more

large yolk-bearing cells in the endoderm after invagination than in the flattened endodermal plate of the blastula. Lwoff points out the insufficiency of the mechanism proposed by Hatschek and claims that other cells beside the large yolk-bearing ones are also turned in, and in this way he accounts for a sufficient number of cells to fill the archenteric cavity. We have also tried to show that, at the dorsal side, cells poor in yolk are invaginated, although we prefer to speak of these cells as endodermal and not as ectodermal, as Lwoff has done.

Hatschek's idea of the method of closure of the blastopore is illustrated by our series of text-figures, VI-X. The dorsal lip is supposed to bend around and meet the ventral lip, thus closing the gastrula mouth along the dorsal side of the embryo. Hatschek offers this view, not in a dogmatic spirit, but simply as more in line with his own observations, admitting, however, that Kowalevski's view may be the correct one. Hatschek noticed the early asymmetry of the gastrula, but a comparison of his Fig. 24 with our Pl. XXXIII, Fig. 2 shows that what he has identified as the dorsal (anterior) lip of the blastopore is, in our estimation, the ventral lip. The older stages, however, are oriented in the same way as are our own. Hatschek noticed that the transition from ectoderm to endoderm is sharpest on the ventral side, and at that point in the later stages he located the two historic pole cells.

It is not entirely clear to us how Hatschek imagined the backward growth of the dorsal lip of the blastopore to take place. His figures lead us to suppose that the result is, in part at any rate, produced by an increase in the number of cells of this region. The ventral wall remains unchanged, and the ventral lip bends around only so far as it takes part in the reduction of the blastopore.

Lwoff draws a sharp distinction between ectoderm and endoderm; the latter cells being characterized by their size and the amount of yolk contained in them, and he believes that the difference is present even in the blastula stage, so that "ehe die Einstülpung beginnt, dass also die Sonderung der zwei primären Keimschichten — des Ektoderms und Entoderms —

hier als Resultat der Furchung zu betrachten ist."¹ He believes the difference has an important practical meaning, for when the invagination is completed, not only the endoderm, but also a portion of the ectoderm is turned in, so that, in the gastrula of *Amphioxus*, not all the cells that line the archenteron are to be designated as endoderm.

Lwoff dissents from Hatschek's statement that at the end of cleavage and before the gastrulation begins all division ceases. On the contrary, Lwoff points out that cell division continues throughout the gastrulation period,—as we have also found,—and that the ectodermal cells divide more frequently than do the endodermal. Lwoff further differs from Hatschek as to the way in which gastrulation takes place. Hatschek states that the endodermal cells are turned in to form the inner layer of the cap-shaped stage and line the entire inner cavity of the cap. Lwoff thinks that the invagination continues during the period of closure of the blastopore. The increase in the ectoderm cells is, according to Lwoff, a most important factor in the gastrulation process. "Auf den Längsschnitten durch die Gastrula sieht man Mitosen überall im Ektoderm, am zahlreichsten aber sind sie an der Seite, die später zur Rückenseite der Larve wird und am dorsalen Umschlagsrande zu bemerken. Man sieht Mitosen auch in den Zellen der dorsalen Wand der Höhle, die sich als eingestülpte Ektodermzellen erweisen. Die Längsschnitte zeigen, dass die Ektodermzellen an diesem Umschlagsrande umbiegen und nach innen wachsen. Das Anwachsen der Zellen muss an dieser Stelle sehr bedeutend sein, weil auf Medianschnitten der Gastrula sich eine Anhäufung von Zellen oftmals bemerken lässt; die Zellen verlieren hier den Charakter des einschichtigen Epithels und sind unregelmässig zweischichtig gelagert. Manchmal lassen sich an der dorsalen Wand der Höhle Unebenheiten bemerken und einzelne Zellen lösen sich sogar aus dem Zellverbande los und erscheinen als rundliche Zellen, die neben den übrigen Zellen liegen. Ich muss hervorheben, dass die Umbiegung der Ektodermzellen und deren Einstülpung nur am dorsalen Umschlagsrande sich bemerken lässt, am ventralen Umschlagsrande dagegen eine scharfe Grenze

¹ Lwoff, p. 5.

zwischen den Entoderm- und Ektodermzellen sichtbar ist. Man könnte freilich den Einwand erheben, dass alle eingestülpten Zellen als Entodermzellen zu bezeichnen sind; aber dieser Einwand könnte nur auf der vorgefassten Meinung beruhen, dass alles, was nach innen gelangt, als Entoderm zu bezeichnen ist. Ich habe schon oben in Uebereinstimmung mit Hatschek angegeben, dass der Unterschied zwischen den Ektoderm- und Entodermzellen schon im Blastulastadium, also vor der Einstülpung, sich bemerken lässt; dieser Unterschied kann seine Bedeutung nicht verlieren, wenn es sich ergibt, dass die Ektodermzellen sich an der Einstülpung auch betheiligen. Die aktive Rolle der Ektodermzellen bei der Einstülpung und die Betheiligung derselben an der Bildung der dorsalen Wand der Höhle kann auf solche Weise keinem Zweifel unterliegen."¹

In regard to the mechanism of the invagination, Lwoff again dissents from Hatschek's view. He shows that Hatschek's account of the enlarging of the endodermal cells is an insufficient explanation in itself, and, moreover, the inturned cells, instead of enlarging, are reduced in size by cell division. We have found that it is difficult to tell how much the endodermal cells increase in volume after each division. A very slight increase in the size of each cell would be sufficient to greatly increase the area of the cell plate, even if the cells themselves do not after division assume their original size. Lwoff's statement that during gastrulation the endodermal cells almost cease to divide is certainly incorrect. Dividing cells are to be found throughout the entire gastrulation period in the endoderm. Some preparations even show only the endodermal cells dividing, and others only the ectodermal. Lwoff has observed the latter only, but an examination of a large number of embryos shows that division takes place in both layers. The number of ectodermal cells is larger than that of the endoderm, so that cell division might be found somewhat more often in the outer layer; but from this it would not follow that any cell of the ectoderm divided more often than any cell of the endoderm. Further, Lwoff's statement that cell division is more abundant on the dorsal side of the blastopore is certainly incorrect, since

¹ Lwoff, p. 7.

our preparations show that cell division is no more frequent here than elsewhere. Lwoff attempts to explain the gastrulation as a result of the more rapid division of the ectoderm at the dorsal lip of the blastopore: "Die Einstülpung beginnt an der Grenze zwischen den Ektoderm- und Entodermzellen, wo der Unterschied zwischen Wachstumsenergien beider Elemente am grössten ist. Da aber die Zellenvermehrung nicht überall gleichmässig vor sich geht, sondern sich vorzugsweise an einer Seite konzentriert, die zur Dorsalseite der Gastrula wird, so erklärt sich dadurch die Ungleichmässigkeit der Einstülpung und die Entstehung einer radial-unsymmetrischen Gastrula. Während nämlich an anderen Stellen die Entodermzellen eingestülpt werden, stülpen sich an dieser Seite die Ektodermzellen selbst nach innen ein. Mit anderen Worten, die Zellen, die vom dorsalen Umschlagsrande aus nach innen wachsen, bilden die dorsale Wand der Höhle, während die eigentlichen Entodermzellen an die ventrale Wand und an die Seiten der Höhle zu liegen kommen. Zugleich wächst der dorsale Umschlagsrand nach hinten, und Hand in Hand damit wird der ursprünglich weite nach hinten offene Gastrulamund allmählich geschlossen. Dadurch kommt eine radial-unsymmetrische, aber zugleich, da die Rückenseite markiert ist, bilateral-symmetrische Gastrula zu Stande, die keineswegs als Archigastrula zu bezeichnen ist."¹

We have shown that Lwoff's statement in regard to the presence of certain cells free from yolk in the dorsal wall of the archenteron is correct. The more recent writers, Klaatsch, Sobotta, and MacBride, have entirely overlooked this important point, and have needlessly criticised Lwoff in consequence. Whether those cells that form the dorsal wall of the archenteron are to be called ectoderm or endoderm is entirely, it seems to us, a matter of choice or definition.

It is the old problem of what we shall define as a germ layer — whether the presence of yolk in certain cells is, in itself, a sufficient criterion to distinguish the endoderm, or whether all the cells that are invaginated are, irrespective of their form, to be called endoderm. For ourselves, the question seems to be a trivial one, and simply as a matter of personal preference we

¹ Lwoff, p. 9.

choose to speak of all the cells that turn in during gastrulation as endoderm.

While we agree with Lwoff that some of the cells that are at first invaginated contain only a small amount of yolk, and clearly resemble the ectodermal cells of the dorsal side, we have found no evidence that the ectoderm continues to turn in at this point during the later period of the closing of the blastopore.

Lwoff, while admitting that Hatschek's figures are true to nature, yet disagrees with Hatschek in regard to the relation of the egg axis to that of the embryo: "Ich habe gefunden, dass der Gastrulamund von allen Seiten geschlossen wird, indem seine Ränder einander entgegenwachsen. Die Schliessung des Gastrulamundes vollzieht sich zwar ungleichmässig, aber ich habe schon gezeigt, dass die Gleichmässigkeit der Einstülpung und der Gastrulaschliessung durch die Einstülpung der Ektodermzellen am dorsalen Umschlagsrande gestört wird, indem, wie oben erwähnt, der dorsale Umschlagsrand während der Gastrulaschliessung nach hinten wächst und mehr als der ventrale und die seitlichen Ränder daran Antheil nimmt. Wenn ich das Wachsthum des dorsalen Umschlagsrandes nach hinten berücksichtige, so könnte ich auch sagen, dass die Schliessung des Gastrulamundes vorzugsweise von vorn nach hinten sich vollzieht, aber nicht in dem Sinne, wie es Hatschek will. Denn ich habe gefunden, dass die Rückenseite der Gastrula selbst nach hinten wächst, dadurch allmählig länger wird und den Gastrulamund schliesst. Während die übrigen Ränder des Gastrulamundes gleichzeitig sich zusammenziehen, wird derselbe immer kleiner. Ich habe dabei keine Spuren der Verwachsung der seitlichen Ränder von vorn nach hinten in der Medianlinie des Rückens (etwa in der Gestalt einer Nathlinie) weder an ganzen Larven noch auf den Schnitten sehen können. Indessen würde die Behauptung, dass der hinterste Theil des Gastrulamundes zuletzt übrigbleibe, nur dann reelle Bedeutung haben, wenn es nachgewiesen wäre, dass die seitlichen Ränder des Urmundes in der Medianlinie in einer von vorn nach hinten fortschreitenden Richtung verwachsen. Diese Behauptung ruht auch auf der Annahme, dass der hintere (ventrale) Rand des

Gastrulamundes während der Gastrulaschliessung unverändert bleibt. Dies ist aber auch nicht der Fall, und Hatschek's eigene Abbildungen sprechen nicht zu Gunsten dieser Annahme."¹

Our view of the method of closing of the blastopore agrees essentially with Lwoff's, that is, that the blastopore closes equally from all sides, and that the gastrula axis corresponds more or less exactly with the longitudinal axis of the embryo. On the other hand, we have tried to show that there is no necessity for supposing that the cells outside the blastopore on the dorsal side turn in during the period of closure of the blastopore. It seems more probable that the advance of the dorsal lip takes place in the same way as that of the lateral and ventral lips, and even Lwoff does not suppose the latter to advance as the result of the inturning of cells.

Wilson made the important observation that "the cleavage pore, which marks the lower pole of the blastula, sometimes persists up to a stage as late as the gastrula shown in Hatschek's Figs. 26 and 27. In all such cases I examined, it lay exactly at the central point of the dome—a fact that shows that the invagination is primarily symmetrical, as originally described by Kowalevski."

Wilson also states, in contradiction to Lwoff's statement, that the entoblastic cells (macromeres) "show numerous conspicuous mitoses, and in every part of the entoblastic plate."²

Klaatsch has given a few figures, mainly optical sections of preserved embryos of *Amphioxus*. He looked for concrescence but failed to find any evidence of it. In regard to the orientation of the embryo he found the closure of the blastopore as described by Kowalevski, "von vorn herein fast genauer aboral und ganz geringe Neigung zur dorsalen Seite hin."³

Sobotta's account follows Hatschek's very closely, and adds little that is new. He states that no distinction exists between the dorsal and ventral wall of the archenteron, during the early gastrula stages, as Lwoff maintained. The explanation of this lies, no doubt, in the preserving fluids that were used. Sobotta states that in the eggs from Naples the blastopore

¹ Lwoff, p. 14.

² Wilson, p. 586.

³ Klaatsch, p. 229.

closes later than in the Messina form, as described by Hatschek. We have had material from both localities and can state that the difference was the result of less normal development in the Neapolitan form. Sobotta has been unable to decide how the axis of the gastrula is related to the axis of the embryo. He believes that the blastopore closes equally from all points and that no concrescence takes place.

MacBride¹ and Sobotta have entirely overlooked the presence of smaller and lighter cells over the dorsal wall of the archenteron, and in consequence MacBride says that "it is difficult to find words to adequately characterize the artificiality and arbitrariness of such a view."

MacBride continues: "If we examine a transverse section of a completed gastrula, . . . we find no difference in character between the cells forming the dorsal wall of the alimentary canal and those forming the ventral wall, such as we should have the right to expect did Lwoff's hypothesis in any way correspond with the facts." This statement is unquestionably wrong, as our figures show.

MacBride gives an inadequate account of the process of gastrulation: "Thus I regard the gastrulation as a fairly uniform pushing in of the under or flattened surface of the blastula, accompanied by division and multiplication of the cells, such multiplication being at first most active in the dorsal (future anterior) lip of the blastopore. The blastopore, which is still wide, becomes rapidly narrowed by the upgrowth of the ventral lip; in contradistinction to what Hatschek asserts, the dorsal lip remains relatively stationary."²

No evidence is offered in support of this opinion, which is, as we have tried to show, incorrect.³

It is not our intention to enter into a comparison of the gastrulation of *Amphioxus* and of the other Chordata. The resemblance of the early larva of the Ascidians to that of *Amphioxus* is, however, so close, that a few words seem justified on this topic. The recent paper by Castle on "The Early Development

¹ MacBride, p. 597.

² *Ibid.*, p. 591.

³ MacBride's Fig. 10 shows the endoderm of the dorsal lip of the blastopore continuing into the ectoderm on the *outer* side of the nerve tube.

of *Ciona intestinalis* " is much more detailed than the work of previous writers and we shall confine ourselves entirely to Castle's account.

In *Ciona*, at the time when the endodermal plate is bending in to form the archenteron (Castle, Fig. 78), a few cells at the dorsal lip of the blastopore also sink in and, in later stages, form the dorsal wall of the archenteron (Fig. 98). From these cells the notochord subsequently develops. They would seem, therefore, to correspond to the cells of the dorsal wall of *Amphioxus*. Outside of the semicircle of notochordal cells (Fig. 62), at the dorsal lip of the early gastrula, lie the ectodermal cells that subsequently form the nerve plate. As the blastopore closes, these cells are carried backward with the advance of the dorsal lip, but none of the cells turn in with the notochordal cells. During the backward growth of the ectodermal and notochordal cells, these cells increase in number. The nervous system of *Ciona* is derived from cells that lie at first in front of the dorsal lip of the blastopore. The nervous system of *Amphioxus* is also derived from cells in front of the dorsal lip of the blastopore, but in *Ciona* the blastopore closes from before backward, while in *Amphioxus*, if our view be correct, the closure is not from before backward, but equally from all points of the periphery. It is important to note that in *Ciona*, in which the advance of the dorsal lip is definitely shown to exist, the ectodermal cells that lie at the free edge of the dorsal lip *do not turn in during the period of closure*.

Castle derives the mesoderm of the tail from a number of cells around the posterior half of the blastopore. These cells are turned into the archenteric space during the period of gastrulation. Castle has followed the lineage of these cells and has shown that they correspond in origin with the ectodermal cells of the anterior region that form the nervous system. He therefore considers them ectodermal in origin, and consequently derives a large part of the mesoderm from the ectoderm. The more anterior mesoderm of the trunk comes from cells lying just within this semicircle of mes-ectodermal cells. This inner circle is described as endodermal in origin. Hence, in *Ciona*,

the mesoderm has a double origin — the duality, however, being a matter of definition. In *Amphioxus* there is nothing indicating that the mesoderm is derived from more than a single source.

In *Ciona* the blastopore closes on the dorsal side, and the longitudinal axis of the embryo seems to be at right angles to the primary or gastrula axis. This would be the case if the dorsal lip grew posteriorly, without the embryo changing shape. On the other hand, there are facts in the development that make it possible to interpret this backward growth of the dorsal lip as the result of a change in the shape of the entire embryo. The gastrula axis, in such a case, would shift during the closure of the blastopore. The shifting would take place in such a way that the dorsal side of the axis is carried backward and the ventral forward. The result would be that the anterior end of *Ciona* would agree with the anterior end of *Amphioxus*. We offer this only as a suggestion, for by this means the orientation of *Amphioxus* and of *Ciona* would be made to agree.

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REFERENCE LETTERS.

<i>a.</i>	anterior.	<i>n.</i>	notochord.
<i>b.c.</i>	body cavity.	<i>op. ect.</i>	opening in ectoderm.
<i>bp.</i>	blastopore.	<i>p.</i>	posterior.
<i>d.</i>	dorsal.	<i>s.c.</i>	segmentation cavity.
<i>ect.</i>	ectoderm.	<i>v.</i>	ventral.
<i>end.</i>	endoderm.	<i>v.p.</i>	vegetative pore.

EXPLANATION OF PLATE XXXIII.

Figs. 1-10, except Figs. 4 *A* and 7 *A*, were drawn with Zeiss 4, oil immersion 2 mm. Figs. 4 *A* and 7 *A* were drawn with Zeiss 4 *D*. The figures in the plate were reduced one-third.

The material from which the figures (except Fig. 6) were drawn was killed in Flemming's solution, the stronger formula. Fig. 6 was drawn from material killed in Hermann's fluid. The section from which Figs. 7 *A*, *B*, *C*, *D*, and Figs. 9 and 10 were drawn was stained in iron haematoxylin. The remaining figures were drawn from unstained material.

FIG. 1. Sagittal section of an embryo at midnight. There is an artificial rupture in the ectoderm; *d.*, dorsal; *v.*, ventral.

FIG. 2. Sagittal section of an embryo at 2 A.M.

FIG. 3. Sagittal section of an embryo at 4 A.M.

FIG. 4 *A*. Oblique section of an embryo at 5 A.M.

FIG. 4 *B*. Enlarged drawing of area marked *x* in 4 *A*.

FIG. 5. Sagittal section of an embryo at 6 A.M.

FIG. 6. Part of a cross-section through the posterior third of an embryo at 8 A.M.

FIG. 7 *A*. Sagittal section through an embryo at 11 A.M.

FIG. 7 *B*. Enlarged drawing of area marked *x* in 7 *A*.

FIG. 7 *C*. Enlarged drawing of area marked *y* in 7 *A*.

FIG. 7 *D*. Enlarged drawing of area marked *z* in 7 *A*.

FIG. 8. Drawn from the anterior region of a longitudinal section of an embryo with eight body cavities; *ect.*, ectoderm; *end.*, endoderm; *b.c.*, body cavity.

FIG. 9. From the anterior wall of an embryo, showing a portion of a cell after division.

FIG. 10. Dividing cell from the endoderm of Fig. 16.

EXPLANATION OF PLATE XXXIV.

Figs. 11-16, and 19, 20, were drawn with Zeiss 4, oil immersion 2 mm. Figs. 17 and 18 were drawn with Zeiss 4 D. The figures on the plate were reduced one-third.

The embryo from which Fig. 11 was drawn was killed in Flemming's solution and was unstained. Figs. 16-18 were drawn from material killed in Flemming's solution, the stronger formula, and stained in iron haematoxylin. The remaining figures were drawn from material killed in corrosive acetic and stained with lithium carmine.

FIG. 11. Blastopore drawn from a total mount at about 2 A.M.

FIG. 12. Blastopore from a total mount at about 3 A.M., showing also endoderm cells through the blastopore opening.

FIG. 13. Blastopore from total mount at about 5 A.M.

FIG. 14. Blastopore from total mount at about 6 A.M.

FIG. 15. Optical section through an embryo at 2 A.M.

FIG. 16. Embryo with endoderm cells turned outward around the vegetative pore.

FIG. 17. Embryo with vegetative pore.

FIG. 18. Embryo with opening at animal pole.

FIG. 19. Embryo, showing vegetative pore at 6 A.M.

FIG. 20. Oblique section of an embryo at 4.30 A.M., showing cells during and after division (at π).